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(54) Title: DEFATTING STARCH CONTAINING WASTE PRODUCTS

(57) Abstract

The invention provides a novel method and use of waste products containing starch and fatty substances. Especially waste food products which do not live up to the manufacturer's specifications are often discarded or used in low economic value applications. For instance, pre-fried French fries are used as cattle feed. The invention allows for these waste products to be used in many applications for which conventionally-obtained starch is applied in that the fatty substances are at least functionally removed from the starch without a significant rise in costs. The fatty substances are functionally removed by an enzymatic treatment employing a lipase.

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Defatting Starch Containing Waste Products

The invention relates to methods for at least the functional removal of fatty substances from products which consist for a major part of starch or starch-like products. In particular, the invention relates to such methods for removing residual fat from food products.

Food products for this invention are defined as any fried, baked, pre-fried or pre-baked edible product which contains both starch (or a starch derivative) and fatty substances such as vegetable oils and fat.

Food products such as (pre-fried) French fries, crisps, chips and other snack foods, cookies, cereals and the like have to meet certain criteria as regards, for example, size, colour and consistency in order to be accepted by consumers. Products which do not comply with set specifications after or during the manufacturing process are either discarded as waste products or recovered to be used in low economic value applications, such as feed for cattle. It would be very useful if these waste or below specification products could be used in applications having a higher economic value.

The starch component of these products (which is usually the major component) could be used as a

carbohydrate s urce in fermentation processes such as for the production of ethanol using yeast or other micro-organisms or the fermentation production of enzymes. However, the manufacturing process of (semimanufactured) food products which are based on starch or starch-like products often includes the use of fatty substances, either as an ingredient or as a (pre-) frying or baking medium.

When such waste products are employed to provide a source of fermentable carbohydrate for fermentation processes, it is found that fatty substances derived from the starting waste product inhibit starch hydrolysis and glucose fermentation when comparison is made to analogous processes employing conventional starch products.

The present invention provides methods for processing of products containing starch and/or starch derivatives and also containing fatty substances wherein treatment is carried out to functionally remove fatty substances. Within the ambit of the present invention are, for example, novel processes for improved use of waste products of the food industry as carbohydrate sources for fermentations, e.g. the fermentation production of ethanol. Such processes are cost-effective and hence attractive.

In one aspect, the present invention thus provides a method of processing a waste product containing as a major component starch and/or one or more starch derivatives and also containing fatty substances to provide a product of

higher economic value, said method including the step of functionally removing fatty substances from said product or a processing derivative thereof by lipase treatment.

The terminology "functionally removing" as used herein will be understood to mean that residual products of the enzymatic breakdown of fatty substances do not need to be physically separated from the starch and/or starch derivative(s) since they do not hamper the use of starch or starch like products in many applications.

The applications of starch are many. Because starch resulting from lipase treatment in a method of the invention is relatively pure, it can be applied in most of them. However, if starch of higher purity is required, this can be easily achieved by separating residual contaminants resulting from enzymatic breakdown of fatty substances using known techniques.

Known applications of starch and starch-like products in which products of methods of the present invention may be employed include, but are not limited to, the food industry (puddings, deserts, bakery products, confectionery, soups, sauces, dressings, snacks, meat products, beverages, milk products, jams and ice-cream), as adhesives (on gummed paper, gummed tape, corrugated board, laminating, bottle labelling, bags, wallpaper, carton sealing, tube winding, etc.), in the paper industry (internal sizing, surface sizing and surface coating), in the textile industry (printing thickening, t xtile

finishing and warp sizing) and many other miscellaneous applications in the pharmaceutical industry, in building materials and the like.

As hereinbefore indicated, methods of the present invention are particularly preferred for providing starch-containing products for use in preparation of fermentation media, especially for use in providing a source of fermentable carbohydrate for fermentation production of ethanol. In this case, lipase treatment will be combined with enzymic hydrolysis of starch and/or one or more starch derivatives.

Lipases suitable for use in a method of the invention may be obtained from bacteria, bacilli, yeasts, fungi or higher eukaryotes. They may be modified chemically or by recombinant techniques, they may be fusion proteins, fragments of enzymes, etc. The only requirement that has to be met is that the enzymatic activity must be present and applicable in the process conditions. Particularly suitable enzymes include the lipase M1 from Pseudomonas pseudoalcaligenes (hereinafter lipase M1), the S80000 lipase from Rhizopus arrhizus and lipase from Mucor miehei. Many other suitable enzymes are known. They include but are not limited to extracellular lipases, such as triacylglycerol acylhydrolases, which are classified in the EEC as E.C.3.1.1.3. These lipases are produced by many (micro)organisms. Suitable microbial lipases have been disclosed inter alia in U.S. Patent no.3,590,277. These

lipases wer obtained from such diverse microorganisms as Pseudomonas, Aspergillus, Pneumococcus, Staphylococcus, Mycobacterium, Humicola, Mycotorula and Sclerotinia. Lipases can be isolated from cultures of these organisms and then used in the methods according to the invention. The lipases can also be produced through recombinant DNA techniques. Some lipase genes have already been cloned. This enables higher production of lipases when microorganisms harbouring these genes are cultured. It also enables the production of more stable or more active muteins of these lipases. All these enzymes can be applied in the methods according to the invention.

In many cases, it may not be necessary to remove all fatty substances to be removed by an enzymatic treatment. When a high percentage of fat is present (in the order of fifteen percent or more), it may be possible and economically advantageous to remove the bulk of fatty substances using another treatment such as a treatment with hot water, e.g. having a temperature of 30-95°C, preferably 50-90°C. Such a pretreatment in combination with lipase treatment as hereinbefore described constitutes another embodiment of the invention.

In the exemplified application of the invention, hot water treatment was not necessary. This is advantageous because of the energy consumed and added costs incurred in carrying out such a treatment.

Waste starch-containing food products are usually not

in a form in which they can be easily subjected to enzymatic treatments. It will therefore usually be necessary to convert such waste products to another form prior to lipase treatment. Preferably, this is a suspension in an aqueous medium. Preferred are aqueous suspensions which contain about up to 50%, most preferably 10-20 % solids (based on dry weight).

Methods according to the invention are preferably applied to fried potato products, more particularly to semimanufactured pre-fried potato products, of which French fries are the most widely used example. French fries are often sold (to consumers or to restaurants or snackbars and the like) as semimanufactured pre-fried products. Such pre-fried French fries contain about 10-20% of fatty substances (usually of vegetable origin). Pre-fried French fries which do not meet the desired specifications (e.g. required food grade quality) will contain about the same amount of fatty substances.

About 70% of these fatty substances can be removed by a pretreatment with hot water, after which the French fries can be cut and suspended in an aqueous medium. As stated before however, pretreatment with hot water may be omitted altogether.

The resulting suspension can then be converted to a source of fermentable carbohydrate by enzymic hydrolysis of the starch present. This may be carried out at a temperature of about 30-70°C and at a pH of about 4-6. The

optimum temperature and pH will, however, depend on the enzyme or enzymes employed.

As shown by results presented below, in this way a source of fermentable carbohydrate can be derived from pre-fried French fries or an alternative starch-containing waste product of the food industry which is far more advantageous for ethanol production by virtue of the decreased inhibition by fatty substances.

The amount in which enzymes have to be added in processing methods of the invention will for a large part depend on the enzyme applied. The person skilled in the art knows how to determine the amount of enzyme needed.

Typically a lipolytic enzyme will be capable of about 2000-5000 conversions per second, which means that when in a kilogram of raw product 10% fat (by weight) is present with an average molecular weight of around 800 D, 2 pg of an enzyme having a molecular weight of 30000 is necessary to break down all the fatty substances in 1 hour. This is of course only a rough estimate of what is possible under ideal conditions.

The following examples illustrate the present invention with reference to one fermenation process for production of ethanol. It will be appreciated, however, that starch-containing products prepared in accordance with the present invention can be applied to many other fermentation methods, including other fermentation methods for production of ethanol from starch or starch-like

products well known in the art.

Examples

Methods

Assay for the determination of lipase activity

Activities of the lipase M1 and lipase of Mucor miehei (Piccantase), expressed as ILUs, were determined on the basis of hydrolysis of olive oil. The hydrolysis was measured at 30°C in a pH-stat containing 10% olive oil in a 0.4 mM Tris buffer pH 9 in the presence of 20 mM sodium chloride and 10 mM calcium chloride.

One ILU is defined as the amount of enzyme needed for the release of one mmole fatty acid per minute under the conditions of the test.

Extraction procedure

After incubation, the remaining triglycerides together with the formed free fatty acids and diglycerides were extracted from the incubation mixture (2 ml) with 5 ml of hexane by rotating the glass tube for 30 minutes after setting the pH at 2 with 4N HCl. The extraction was terminated by centrifugation for 5 minutes at 1000 rpm in a table centrifuge to obtain a clear upper layer containing triglycerides, diglycerides and free fatty acids.

HPLC analyses

In the extraction solution, the residual amount of triglyceride together with the amount of diglyceride and free fatty acid formed were determined by HPLC.

Equipment and conditions

Pump:	LKB (model 2150)
Detection:	Refractive index monitor (Jobin Yvon)
Injection system:	Perkin-Elmer ISS-101; 10 ml
Integrator:	Spectra Physics, Chromjet
Column:	CP Microspher-Si (Chrompack), 100 x 4.6 mm
Eluent:	n-hexane/isopropylalcohol/formic
Acid:	975/25/2.5 (v/v), 1 ml/min
Temperature:	ambient

Under the conditions described above, the ratio of the refractive index responses between olive oil, oleic acid, 1,2 and 1,3-diacylglyceride were found to be 1.00, 0.98, 2.10 and 1.30, respectively, on the basis of peak height.

EXAMPLE 1

Lipase Treatment of an Aqueous Suspension of Pre-Fried French Fries

Pre-fried French fries (fried in vegetable oil) were obtained in frozen form. After thawing, the fries were cut into small pieces and a 20% (based on dry weight) suspension was made in a 100mM MES buffer pH 5.5 with a Waring commercial blender.

Incubations were performed at 50°C over different time periods and with different lipase dosages, as indicated in Table 1. The lipases used were the lipase M1 of Pseudomonas pseudoalcaligenes and lipase from Mucor-miehei. Both lipases are products of Gist-Brocades and are commercially available. After extraction of the remaining triglycerides and hydrolysis products with hexane, these products were analysed by HPLC as described hereinbefore. The results are summarized in Table 1.

TABLE 1

Percentage breakdown of vegetable oil from pre-fried French fries upon lipase treatment.

lipase	dosage (ILU/ml)	% breakdown after indicated incubation time (hrs) 0.5	1.0	2.0	4.0	16
--	0	3	3	3	3	2
Lipase Ml	2	5	8	12	13	41
"	4	7	9	12	19	49
"	10	10	14	24	32	44
"	20	10	17	30	49	60
"	40	13	25	41	58	63
Mucor miehei	2	4	4	5	9	9
"	4	4	5	6	10	10
"	10	4	5	6	8	12
"	20	6	8	10	18	12
"	40	5	6	8	12	16

From this table, it can be seen that the lipases used showed their lipolytic properties on vegetable oil under the conditions investigated. In comparison to the Mucor miehei lipase, lipase Ml was found to be superior.

EXAMPLE 2

Ethanol Production by Fermentation Using Fermentable Carbohydrate Derived From Pre-Fried French Fries

Pre-fried French fries were chopped into small pieces with a blender. The dry solids content of the material was determined by oven drying overnight at 70°C. Starch content was measured using the Megazyme® Starch Kit as commercialized by Megazyme®. Enzyme dosages were determined based on dry solids (33%) and starch content (75%).

Three 900 ml mash portions were set up in identical 1l bottles each containing an emulsion in water of 20% French fries (based on dry weight), as prepared in a blender. The emulsion was adjusted to pH 5.5 with 6N HCl and 6N NaOH as required. Two bottles were treated with lipase Ml (500 grams/ton based on dry weight of French fries) for 4 and 16 hours in a 50°C waterbath. The third bottle was a control without lipase pretreatment.

After lipase pretreatment, all three bottles were adjusted to pH 6.5 with 1N NaOH and transferred to a 60°C waterbath and held for 30 minutes to equilibrate the temperature prior to alpha amylase (Maxaliq® ET) addition in a dosage of 0.7 l/ton based on dry solids starch (ds starch; 1 ton = 1000 kg). After mixing for 5 minutes, the samples were autoclaved for 5 minutes at 107°C. Then the bottles w re returned to the waterbath, where they wer

h ld at 93°C for an additional 2 hours. During this incubation, the samples were stirred every 15 minutes.

After liquefaction, the samples were cooled to 60°C, adjusted to pH 4.8 and transferred to sterile 500 ml media bottles. Fermentations were performed in triplicate. Additional enzymes [Amigase® (0. 8 l/ton ds starch), Mycolase (0.07 l/ton ds starch), Maxazyme® NPX (0.2 l/ton ds starch)] were added and thoroughly mixed with the mash prior to yeast addition. Fermiol was added at the concentration of 0.2 kg/ton of mash. Penicillin was added at the concentration of 500 units/ l of mash.

All nine media bottles were transferred for fermentation over 63 hours to a shaker bath adjusted to 33°C/150 rpm. Samples were taken at 15, 24, 40,48 and 63 hours and analyzed by HPLC (Bio-Rad HPX-87H column, RI detector) for total carbohydrates, fermentable carbohydrates and ethanol.

HPLC results of ethanol production are shown in Table 2. Throughout the fermentation, the lipase treated French fries produced a higher ethanol yield. Residual total and fermentable carbohydrates are shown in Table 3. As expected from the observed ethanol yields, there was a large proportion of both fermentable and total carbohydrates in the control sample at the end of fermentation. Ethanol yields were calculated at 63 hours (grams ethanol produced/grams of potential ethanol based on ds starch). Control samples produced an average yield of 77%, compared

to an average yield of 90% and 92% respectively for the 4 and 16 hour lipase pre-treated material.

This example shows clearly that lipase pre-treatment of pre-fried French fries results in higher ethanol yields in fermentation.

TABLE 2

Ethanol profile during a 63 hour fermentation

Time	Ethanol produced (volume percent)		
(hours)	control	lipase treated (4hrs)	lipase treated (16 hrs)
0	0. 00	0. 00	0. 00
15	3. 59	5. 80	5. 33
24	5. 84	7. 42	7. 70
40	6. 96	8. 38	8. 54
48	7.79	8. 92	9. 06
63	8. 29	9. 71	9.92

TABLE 3

Residual total and fermentable carbohydrates.

sample	residual carbohydrates after 63h (weight %)	
	total	fermentable
Control	3.0120	2.1920
lipase treated (4hrs)	1.4600	0.6900
lipase treated (16hrs)	0.7614	0.0764

From this experiment another unexpected advantage becomes clear. The amount of residual fermentable carbohydrates was lower when a lipase-treated aqueous suspension of French fries was employed. This will lead to a decreased burden on the environment since the wasteload of the reactor residue is lower.

CLAIMS

1. A method of processing a waste product containing as a major component starch and/or one or more starch derivatives and also containing fatty substances to provide a product of higher economic value, said method including the step of functionally removing fatty substances from said product or a processing derivative thereof by lipase treatment.

2. A method as claimed in claim 1 wherein said waste product is a waste product of the manufacture of one or more food products.

3. A method as claimed in claim 1 or claim 2 wherein a lipase is contacted with an aqueous suspension derived from said waste product under conditions suitable for hydrolysis of fatty substances in said suspension.

4. A method as claimed in claim 3 wherein said aqueous suspension contains 10 - 20% solids (based on dry weight).

5. A method as claimed in claim 3 or claim 4 wherein said aqueous suspension is maintained at a temperature of about 50°C during lipase treatment.

6. A method as claimed in any one of claims 3 to 5

wherein said aqueous suspension is derived from one or more pre-fried potato products.

7. A method as claimed in claim 6 wherein said aqueous suspension is derived from pre-fried French fries.

8. A method as claimed in any one of the preceding claims wherein lipase M1 of Pseudomonas pseudoalcaligenes is employed for said lipase treatment.

9. A method as claimed in any one of the preceding claims wherein said lipase treatment is preceded by heated water extraction of a proportion of the fatty substances from said product or a processing derivative thereof.

10. A method as claimed in any one of the preceding claims wherein said waste product is converted to a source of fermentable carbohydrate, said method further comprising enzymic hydrolysis of starch and/or one or more starch derivatives deriving from said waste product.

11. A method as claimed in claim 10 wherein hydrolysis of starch and/or one or more starch derivatives is preceded by said lipase treatment.

12. A method as claimed in claim 11 wherein said lipase treatment is applied to an aqueous suspension of one or

more pre-fried potato products in accordance with claim 6 followed by α-amylase digestion of the starch in the resulting suspension.

13. A method of preparing a fermentation medium comprising preparing a source of fermentable carbohydrate by a method as claimed in any one of claims 10 to 12 and incorporating said source of fermentable carbohydrate with other appropriate ingredients into a complete fermentation medium.

14. A method as claimed in claim 13 wherein said fermentation medium is suitable for yeast fermentation production of ethanol.

15. A method for production of ethanol, wherein a fermentation medium is prepared in accordance with claim 13 or claim 14 and fermentation is carried out to convert fermentable carbohydrate in said medium to ethanol.

16. Use of an enzyme having lipolytic activity in a method as claimed in any one of the preceding claims.